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Neurobehavioral Effects of Triadimefon, a Triazole Fungicide, in Male and Female Rats

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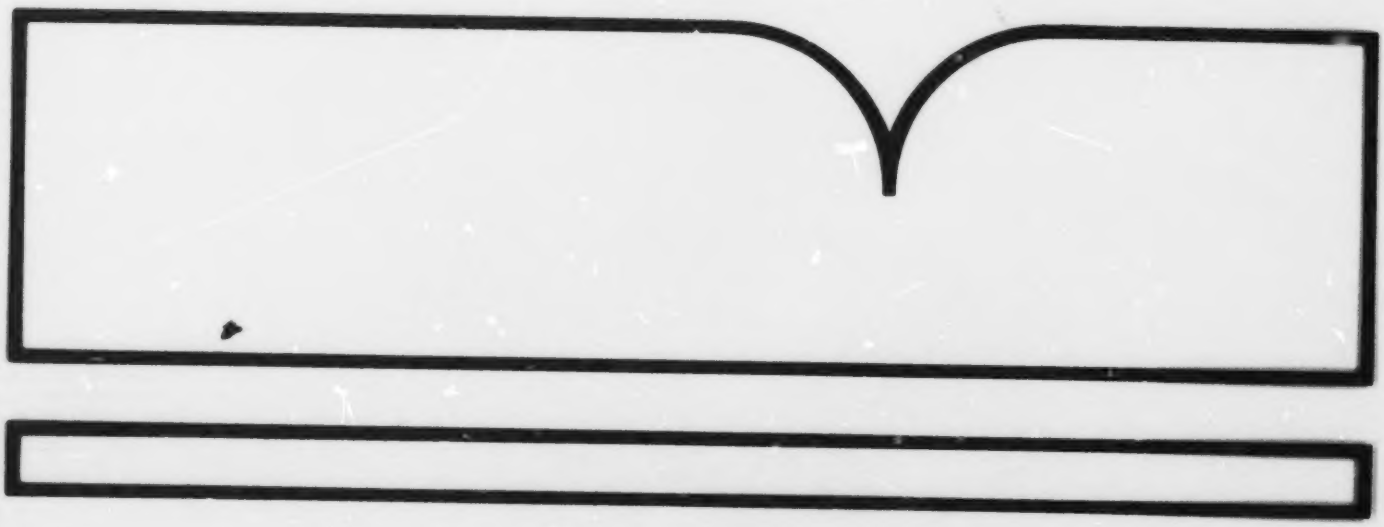

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16. ABSTRACT

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Neurobehavioral Effects of Triadimefon, a Triazole Fungicide, in Male and Female Rats[1]

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MOSER, V. C. AND R. C. MACPHAIL. *Neurobehavioral effects of triadimefon, a triazole fungicide, in male and female rats.* NEUROTOXICOL TERATOL **11**(3) 285–293, 1989.—Triadimefon is a widely used systemic fungicide, yet there is little published information on its effects in mammals. This study describes the effects of triadimefon in male and female rats using a functional observational battery (FOB), motor activity (measured in a figure-eight maze), and operant performance (responding under a fixed-interval 3-min schedule). For the FOB, Long-Evans hooded rats were tested immediately before dosing and 0.5, 4, 24, and 48 hr after IP dosing with either vehicle, 30, 100, or 300 mg/kg triadimefon. Prominent effects of triadimefon (100 and 300 mg/kg) included increased arousal, stereotypies involving repetitive sniffing, head bobbing, and pacing, and self-mutilation. Dose-related handling-induced convulsions, changes in reflexes and sensory reactivity, hypothermia, and body weight loss were also significant findings. Doses of 30, 75 and 150 mg/kg triadimefon increased figure-eight maze activity whereas 300 mg/kg decreased activity. Habituation of activity during the session as well as the spatial distribution within the maze were also affected by triadimefon. Overall rates of responding maintained by fixed-interval milk reinforcement were increased at 30 and 56 mg/kg, and decreased at 100 and 200 mg/kg. Responding within the 3-min fixed-interval was also affected, with low rates normally occurring early in the interval markedly increased. These effects on operant performance were similar to those seen following *d*-amphetamine, and were attenuated by pretreatment with chlorpromazine (0.5 mg/kg). On many measures, female rats appeared to be somewhat more sensitive than males. Recovery was evident in some measures the day after dosing, but the effects of high doses (≥100 mg/kg) were typically prolonged (several days). Thus triadimefon produced a unique neurotoxic syndrome which is similar in many aspects to that produced by CNS stimulants.

Triadimefon Triazole fungicide Neurotoxicity Motor activity Operant performance Functional observational battery Rats

TRIADIMEFON (1-(4-chlorophenoxy-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone) is a widely used triazole which is fungicidal via inhibition of ergosterol biosynthesis, and is systemically effective against powdery mildew and rust diseases on fruits, grains and shrubs (1, 2, 24). There are very few reports on the effects of triadimefon in mammals. Acute oral LD_{50} values are 568 mg/kg (males) and 363 mg/kg [females; (19)] and the LD_{50} determined in this laboratory in adult male rats was 812 mg/kg (23). Triadimefon and its metabolite, triadimenol, has been shown to increase motor activity of male mice and male rats (6,17). This stimulant-like action of triadimefon on motor activity was attenuated in male rats by pretreatment with reserpine but not with α-methyl-para-tyrosine (5), suggesting that triadimefon produces this effect by interacting with reserpine-sensitive neurotransmitter pools in the CNS.

The stimulant-like actions of triadimefon on motor activity appeared to be unique in pesticide research, and suggested that a more thorough characterization of its neurobehavioral effects would be of great value. We therefore studied the effects of triadimefon using schedule-controlled operant behavior, motor activity, and a functional observational battery (FOB). Collectively these tests offer the advantage of assessing a variety of neurobehavioral functions involving innate and acquired behaviors and have been recommended for evaluating effects of environmental chemicals (32). In addition, all tests are noninvasive and can be applied repeatedly to establish a profile of neurobehavioral effects, including time-course and dose-response characteristics for triadimefon. We used both male and female rats in order to examine possible sex-related differences. The profile of effects that was established provides strong evidence for the similarity of triadimefon's effects to those of amphetamine-like CNS stimulants.

METHOD

Animals

Adult Long-Evans hooded rats of both sexes (Charles River

[1]The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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TABLE 1

OVERALL SUMMARY OF TRIADIMEFON EFFECTS IN THE FOB

	M	F		M	F
Autonomic			Muscle Tone, Equilibrium		
Defecation	↓ **	↓ **	Gait	**	**
Urination	↓ **		Forelimb grip strength		
Home cage palpebral closure	*		Hindlimb grip strength		
Pupil response	*	**	Righting reflex	*	*
Salivation			Hindlimb foot splay		↓ *
Piloerection	*	*	Extensor thrust		
Exophthalmus			Limb rotation	↓ *	
Lacrimation	*	*			
CNS Activity, Excitability			Sensorimotor		
Rearing	↓ **	↓ **	Tail pinch response	↓ ↑ *#	↓ *
Arousal	↓ ↑ *#	↓ ↑ *#	Approach response		↓ *
Home cage posture	**	*	Finger snap response	↓ **	↓ **
Removal difficulty		↑ **	Touch response		↓ *
Handling difficulty		↑ **	Catalepsy		
Convulsions	*	*	Handling palpebral closure	*	
Latency to first step					
Fur appearance	*	*	Physiologic		
Crusty eyes	**	*	Temperature	↓ **	↓ **
Vocalization					
Circling	**	**	Weight	↓ *	↓ *
Biting	*	*			
Stereotypy	**	**			

*Affected only at highest dose.**
†Dose-dependent, affected at ≥2 doses.
↓ ↑ Indicates direction of change where applicable.
#Effect at lowest or middle dose only.
Measures are grouped into categories to represent functionality.
Dose-response data are indicated, collapsed across time points.

Laboratories, Raleigh, NC) were used in all experiments and were at least 70 days of age when testing began (males approximately 350–400 g, females 240–270 g). Rats were singly housed in wire mesh cages and in most instances allowed free access to Purina rodent chow (Rat Chow No. 5002; Ralston Purina Co., St. Louis, MO) and deionized tap water. For the operant experiments, rats were maintained at 350 g body weight (males) or 240 g (females) by limiting daily food intake. The animal facility was maintained at 22 ± 1°C, 55 ± 5% humidity, and had a 12 hr light:dark cycle with lights on at 0700 hr. FOB testing took place in the animal housing room, while for the motor activity and operant behavior experiments rats were transported to other, isolated rooms in the animal facility.

Experiment 1—Functional Observational Battery

Procedure. The FOB procedure which was used herein, is described in full detail elsewhere (21). First, the observer described the posture, activity, and presence of convulsions in the rat in its home cage. The rat's reactivity to removal from the cage and handling were then rated, and the observer noted and/or ranked the presence of salivation, lacrimation, piloerection, ptosis, exophthalmus, and the general state of fur appearance. Next, the rat was placed on an open field (the top of a 60 × 90 cm laboratory cart) for 3 min, during which time the activity level and gait characteristics were scored and the number of rears, fecal boluses, and pools of urine were counted. The presence of convulsions was again noted. Reflex testing included each rat's response to the approach of a pencil, a touch to the rump, finger snap, tail pinch using forceps, and its pupillary response to a penlight stimulus. Extensor thrust, limb rotation, degree of catalepsy, and righting reflex were rated next. Forelimb and hindlimb grip strength was measured using strain gauges, and the hind feet were then painted lightly to measure landing foot splay. Finally, the rat was weighed and the rectal body temperature was taken. Tests were completed in approximately 6–8 min per rat. All data were recorded on standardized sheets and later entered into a computer for further analysis.

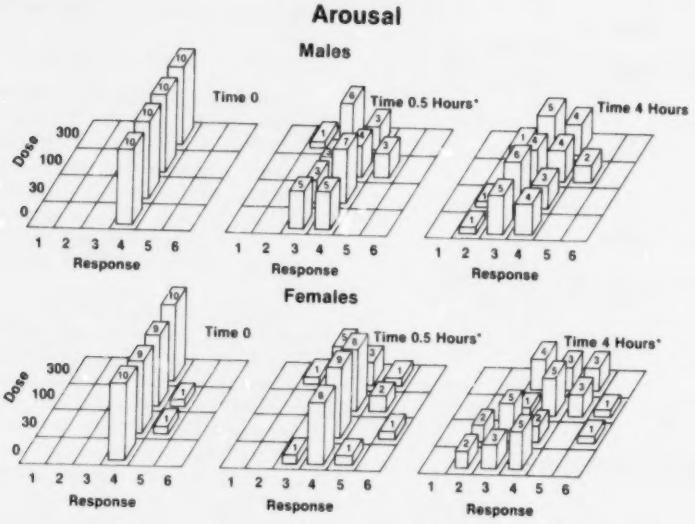

FIG. 1. Effects of triadimefon on arousal. The rating scale ranges from 1 to 6—from very low, or stupor, to very high, or sudden darting and hyperalert. Numbers on top of bars indicate the number of rats given each score in each dose group. Time points at which there was a significant difference between dose groups are indicated (*). Data for 24 and 48 hr not included for clarity. N = 10/dose/sex.

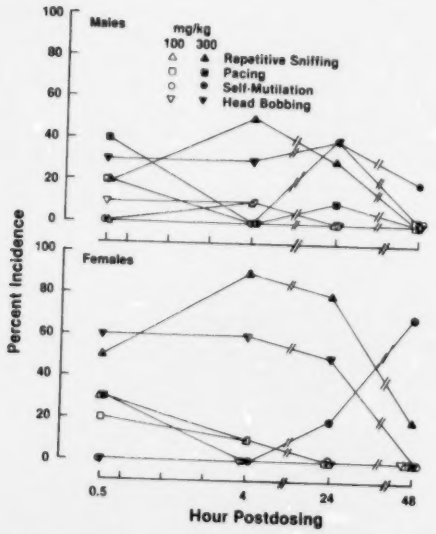

FIG. 2. Incidence and time course of various stereotypies produced by triadimefon 100 mg/kg (open symbols) and 300 mg/kg (filled symbols). Descriptions of these signs are in the text. Baseline frequency of these signs are zero. N = 10/dose/sex.

Ten rats of each sex were treated IP with either vehicle or 30,100, or 300 mg/kg triadimefon. The battery of tests was administered to each rat immediately prior to dosing (time 0) and at 0.5, 4, 24, and 48 hr after dosing. The observer was blind with respect to each rat's treatment.

Data analysis. The analysis of procedures for the FOB data are described in Moser *et al.* (21) and Creason (4). Two-way ANOVAs were carried out using a grouping factor of dose and repeated measures across time. Continuous data such as body weight and grip strengths were analyzed by a linear model [GLM; (29)] using each rat's time 0 value as a covariate. Descriptive and rank data (scores such as reflexive responses, gait descriptions, etc.) were analyzed using a categorical procedure [CATMOD; (29)] Univariate analyses at each time point were performed when there was a significant dose effect or dose-by-time interaction. Only statistical tests with resulting p-values<0.05 are reported as significant.

Experiment 2—Motor Activity

Procedure. Motor activity was measured in a maze composed of a series of interconnected alleys shaped like a figure-8 with two blind alleys projecting from the central arena [see (27)]. Six phototransmitter/diode pairs were equally spaced around the figure-eight portion of the maze, and one pair was located in each of the blind alleys. Motor activity was recorded by a microprocessor as photocell interruptions, in successive 5-min intervals of a 1-hr session for analysis of the temporal distribution (habituation) of activity during the session. In addition, individual photocell interruptions were totaled for analysis of the spatial distribution of activity (i.e., whether it occurred in the figure-eight portion or in the blind alleys).

Eight rats of each sex were dosed with either vehicle, 30, 75, 150 or 300 mg/kg triadimefon and tested 20 min, 24 hr, 48 hr and 144 hr (6 days) afterward; a noninjected control group was also included.

Data analysis. Total photocell counts on each day of testing were analyzed by a two-way ANOVA with sex and dose as the independent variables (29).When there was a significant main

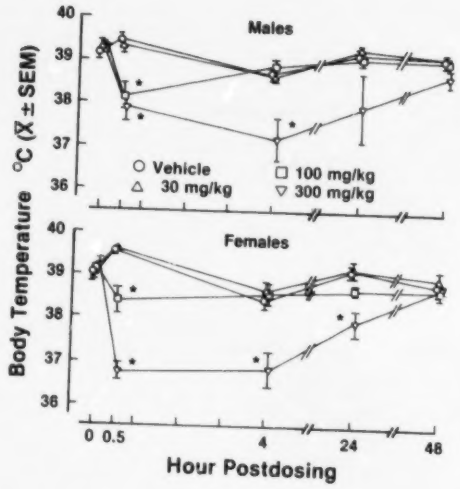

FIG. 3. Effects of triadimefon on body temperature (mean ± S.E.M.). Data significantly different from control are indicated (*). N = 10/dose/sex.

effect or interaction, simple-effects ANOVAs were carried out for each sex followed by Dunnett's *t*-test to determine which dose groups differed significantly from noninjected control values. Both the interval data (counts per 5-min interval) and spatial data (counts in each area divided by the number of photocells in each area—six in the figure-eight portion and two in the blind alleys) were analyzed by an ANOVA with grouping factors of dose, sex, and repeated measures on the interval or spatial data. Greenhouse Geisser probabilities were used for all ANOVAs (14); $p<0.05$ was accepted as significant. Mean contrast comparisons were performed using Tukey's studentized range test (29).

Experiment 3—Schedule-Controlled Performance

Procedure. Operant testing took place in commercial operant chambers (Model E10-10, Coulbourn Instruments Co., Lehigh Valley, PA) located inside larger ventilated, sound- and light-attenuating chambers. The front panel of the chambers contained a response lever, a dipper magazine, and a centrally-located houselight. The schedule contingencies and data collection were controlled by a superSKED software system (State Systems, Inc., Kalamazoo, MI) with a PDP8/E minicomputer (Digital Equipment Corp.).

Eight male and six female rats were trained Monday through Friday to lever press under a fixed-interval (FI) 3- min schedule of milk reinforcement. Under this schedule, the first response after 3 min, but before 4 min (60 sec limited hold), was reinforced with a 4-sec delivery of 0.03 ml of milk (one part Borden's Eagle Brand sweetened condensed milk: two parts water). A 4-sec time-out period followed delivery of each reinforcer or expiration of the limited hold, during which the chamber was dark and responses had no programmed consequences. The houselight was illuminated throughout the session except during the time-out, and during milk delivery when a light above the milk dispenser was on. Each session lasted for 12 intervals, and under control conditions rats received all reinforcers within 36 to 38 min.

For dose-effect determinations, rats were administered either vehicle, 3, 10, 30, 56, 100, or 200 mg/kg triadimefon IP 30 min before the session in a semirandom order, except that the highest dose was given last. Effects of 10 and 56 mg/kg were determined twice, and included in the data analysis as separate treatments. Triadimefon or vehicle was given ordinarily on Tuesdays and Fridays, and performance on Thursdays provided control data. A week or more separated the testing of the high doses of triadimefon (≥56 mg/kg), due to prolonged side effects.

Data analysis. Overall response rates, response rates in each tenth of the fixed-interval (local response rates), and index of

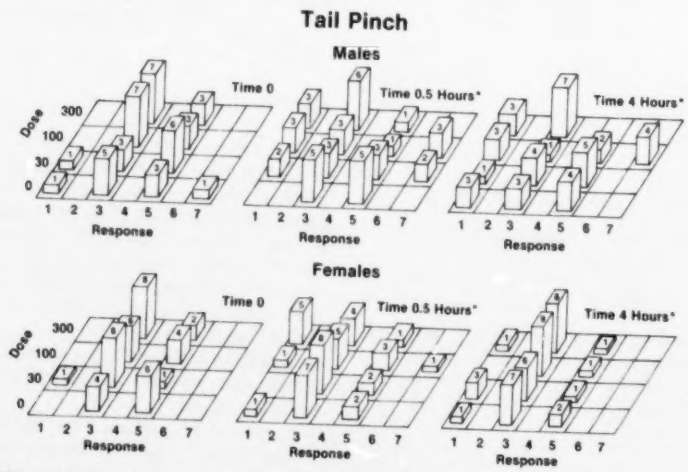

FIG. 4. Effects of triadimefon on the tail pinch response. Descriptions of the response categories are [summarized from (21)]: 1) no reaction, 2) rat slowly turns, 3) rat moves away, 4) rat freezes, 5) rat quickly turns, 6) rat jumps away, 7) extreme, rapid reaction. Numbers on top of bars indicate the number of rats given each score in each dose group. Time points at which there was a significant difference between dose groups are indicated (*). Data for 24 and 48 hr not included for clarity. N = 10/dose/sex.

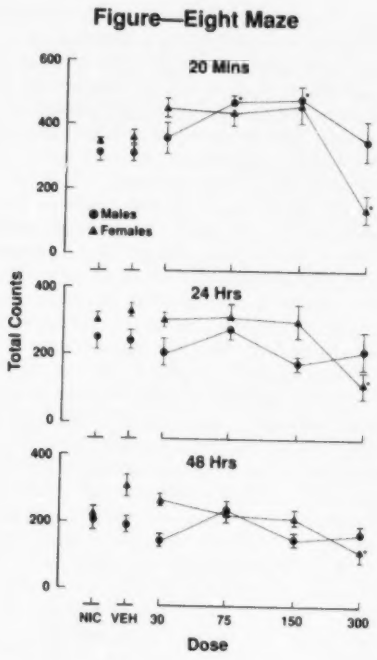

FIG. 5. Effects of triadimefon on motor activity in the figure-eight maze (mean total photocell counts in 1 hr session ± S.E.M.) 20 min, 24 hr, and 48 hr after dosing. Data significantly different from control are indicated (*). N = 8/dose/sex.

curvature (IOC) values were calculated for each rat's session. IOC is a measure of the extent and direction of the temporal pattern of responding [see (12)]; a value approaching 1 indicates increased responding throughout the interval.

IOC values were not included if the corresponding overall response rates were <0.01 response/sec, since it becomes an inaccurate measure of response patterning if only a few responses are emitted. For graphical purposes, treatment effects on response rates were calculated as a percentage of each subject's mean control value, and then averaged across all subjects. Statistical analyses were performed on the actual values. Response rates in each tenth of the interval were averaged across subjects and rate-rate plots were constructed [see (3)] relating the log of the local rates at each dose as a function of the corresponding log control rates.

Overall response rates and IOC values were analyzed by repeated-measures ANOVA for both sexes, followed by Dunnett's *t*-test to determine doses at which group means differed significantly ($p<0.05$) from noninjected control. IOC data for doses which severely or completely suppressed responding (100 and 200 mg/kg) was limited, since IOC values could not be determined for many of the rats. The available data was thus compared to control data by a Student's *t*-test with a Bonferroni correction of the α level (14).

Probe experiments. Following recovery from the effects of 200 mg/kg, several probe manipulations were arranged to explore possible mechanisms of triadimefon's action. Rats were first administered doses of *d*-amphetamine SO_4 (1 and 2 mg/kg) 20 min presession. Secondly, rats were given an injection of either chlorpromazine HCl (0.5 or 0.75 mg/kg) or saline at 30 min, followed by triadimefon (10, 30 or 56 mg/kg) or vehicle at 20 min

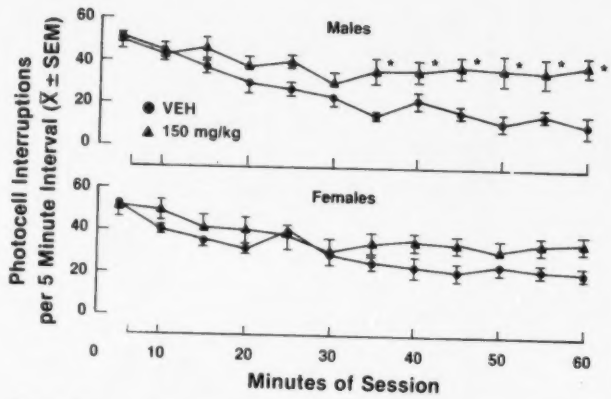

FIG. 6. Effects of triadimefon on motor activity (mean photocell counts ± S.E.M.) in 5-min intervals of the 60 min session, 20 min following triadimefon 150 mg/kg. Data significantly different from control are indicated (*). N = 8/dose/sex.

presession. N = 7 for male rats, since one rat displayed extreme self-mutilation following triadimefon 200 mg/kg in the dose-response study, and had to be sacrificed.

Chemicals

Triadimefon (99.0% purity) was obtained from Chem Service Inc. (West Chester, PA) and suspended in 5% ethanol, 5% Emulphor (EL-620; GAF Corp., Charlotte, NC) and saline. A fresh suspension was made every 2–3 days. Control groups were treated with a vehicle of the same proportions of ethanol, Emulphor, and saline. Chlorpromazine HCl and *d*-amphetamine SO_4 were obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in saline shortly before dosing. All injections were administered IP in a volume of 1 ml/kg.

RESULTS

Experiment 1—Functional Observational Battery

The various measures of the FOB have been grouped into categories to present a profile of the effects on various functional systems [see Moser (20)]. Table 1 presents a summary of the effects of triadimefon in each category, regardless of the time point at which the effects occurred. Many of the effects were clustered in the general category of CNS activity and excitability, with more than half of the tests in this category being affected by at least two doses of triadimefon in one or both sexes. Triadimefon also decreased body temperature and weight in both sexes. Sensory affect was disrupted somewhat, as well as autonomic function and equilibrium. Muscle tone was least affected.

Triadimefon affected most of the measures which assess spontaneous activity. The effects of triadimefon on arousal (level of unprovoked activity) are presented in Fig. 1. A biphasic response was observed in both sexes, with more rats displaying increased arousal (rankings of 5 and 6) at 30 and 100 mg/kg, and hypoactivity and lethargy (2 and 3) at 300 mg/kg. In males, arousal scores were significantly different from the vehicle group only at 0.5 hr following 100 and 300 mg/kg and 48 hr later (highest dose only). On the other hand, females showed only decreased arousal at 300 mg/kg 0.5 hr after dosing, and at 4 hr higher arousal scores were noted at 100 and 300 mg/kg. The

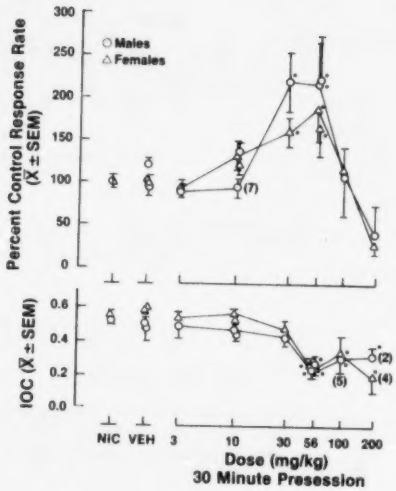

FIG. 7. Effects of triadimefon on operant performance 30 min after injection. Top panel shows effects on response rate (mean % control ± S.E.M.), bottom panel shows index of curvature values (mean ± S.E.M.). Data significantly different from vehicle control values are indicated (*). N = 8 males, N = 6 females, except where numbers in parentheses are different. NIC = noninjected controls; extra datapoints at 10 and 56 mg/kg are replications.

number of unsupported rears, or rears made away from the edge of the cart, were decreased in males by 100 and 300 mg/kg at 0.5, 4, and 48 hr; 30 mg/kg was effective as well at 24 hr. In females, this decrease was obtained at 100 and 300 mg/kg 0.5 hr after dosing, at all doses by 4 hr, and only at 300 mg/kg 24–48 hr later. An increase in reactivity in removal and handling was noted only in females at 0.5–4 hr after dosing (all doses affected ease of removal, and 100 and 300 mg/kg affected ease of handling). At 0.5 hr after the highest dose, rats (20% males, 80% females) displayed a range of moderate to severe whole-body clonic convulsions. In two cases (males) these alternated with emprosthotonus—tonic seizures of the flexor muscles in which limbs and head are curved forward. The convulsions were not observed in the home cage in most of the rats, and thus may have been evoked by handling.

A remarkable feature of triadimefon exposure involved stereotypies and autotomy (self-mutilation), as summarized in Fig. 2. In the home cage, rats displayed head bobbing (head repeatedly nodding up and down or from side to side) 0.5 to 24 hr after dosing. In the open field,the most prominent action was repetitious sniffing in one area. The incidence of head bobbing and sniffing was greater in females, and generally lasted longer (up to 48 hr). Stereotyped circling around the perimeter of the cart was also observed primarily on the day of dosing. Autotomy was obvious following 300 mg/kg in both sexes, though more pronounced in females, at 24 to 48 hr after dosing. The intensity of self-mutilation ranged from scabs and sores on the digits to instances in which the bone and flesh were revealed and paws were chewed off. Rats so severely affected had to be sacrificed. Under our conditions of testing, rats normally groom very infrequently. However, at 300 mg/kg, 50% of males at 4 hr, and 60% of females at 48 hr displayed two or more episodes of stereotypic grooming.

The general appearance and health of the rats were also markedly affected by the highest dose of triadimefon. The fur of most rats was soiled (up to 60% males and 80% females, 4–48 hr after dosing), and 10–30% of the rats showed dried crust around the eyes. Piloerection was also evident in 50–60% of the rats along with slight ptosis in 20–40% of male rats, at all time points after the highest dose. Body weight loss was evident from 4 to 24 hr, and at 48 hr the rats were no longer actually losing weight but were still lower than control rats. Maximum weight loss for male and female rats was about 30 g and 24 g, representing 8 and 9% of the weights, respectively, at time 0. Body temperature was decreased in a dose-related manner in both sexes, and is shown in Fig. 3. This hypothermia was evident at 0.5 hr and lasted 4 hr in males, and 24 hr in females.

Responses to all stimuli were depressed in females, from 0.5 to 24 hr after the highest dose. An exception was the finger-snap response, which was decreased by 100 and 300 mg/kg but only at 0.5 hr after dosing. In contrast to the general reflex depression seen in females, only the tail pinch and finger-snap responses were affected in males. As with the females, decreased reflexes were recorded in response to the finger snap, evident at all doses at 4 hr and only at 300 mg/kg at 24 hr. Figure 4 shows biphasic responses that were obtained in males following the tail pinch, and the decreased responses at the highest dose in the females. In males, there were more hyperreactive responses (7's) as well as some dulled responses (1's) at all doses at 0.5 hr. This was still evident at 100 mg/kg at 4 hr, but only response depression, which lasted

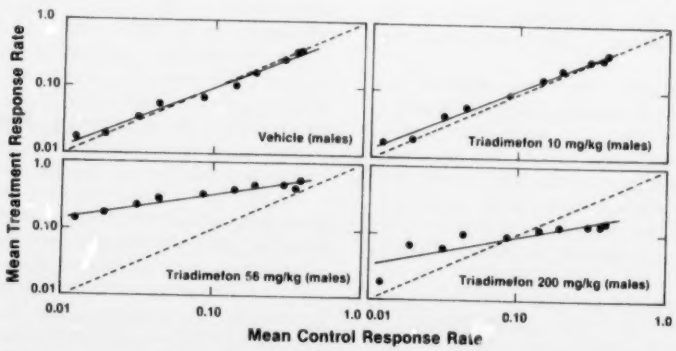

FIG. 8. Mean rates of responding (response/sec) in successive tenths of the interval following various doses of triadimefon plotted as a function of corresponding mean local response rates under control conditions. The dashed diagonal represents the line of no effect (slope = 1).

to 24 hr, was seen at the high dose.

Triadimefon affected few autonomic functions. Defecation (both sexes) and urination (males only) were decreased by the two higher doses at 0.5–4 hr, and by 300 mg/kg at 24 and 48 hr. Lacrimation (10–40% incidence) was produced by the highest dose at 4–48 hr. The pupil constriction response was inhibited by 100 (females only) and 300 mg/kg (both sexes). On this measure a delayed peak effect of 300 mg/kg, 4 hr as opposed to 0.5 hr for 100 mg/kg, was obvious in females. A 10–40% incidence of mydriasis was also noted in both sexes at 100 and 300 mg/kg, with the peak effect occurring at 4 hr.

Equilibrium was disrupted by triadimefon, as measured by righting reflex and gait. Whereas righting was impaired only 0.5 hr after 300 mg/kg, altered gait (primarily ataxic and hunched) was seen at all doses on the day of dosing and at 24–48 hr following the highest dose. Muscle tone was only affected by the highest dose as measured by landing foot splay (females only, at 24 hr) and limb rotation (males only,at 24 hr).

Experiment 2—Motor Activity

General effects of triadimefon noted in these rats were similar to those described for the FOB, including stereotypies, convulsions, self-mutilation, and weight loss, primarily after 300 mg/kg. One female dosed with 300 mg/kg died approximately 5 days after dosing, and this was the only death observed after triadimefon. Decreased body weights in the highest dose group had returned to or surpassed preinjection values by 6 days after dosing.

Triadimefon produced a significant effect on motor activity on the day of dosing, and 24 and 48 hr afterward (Fig. 5). At each of these timepoints, there was a significant dose-by-sex interaction and thus the simple effects were analyzed separately for each sex. On the day of dosing, an inverted U-shaped curve was obtained for males, with significant increases in activity levels after the intermediate doses (75 and 150 mg/kg). Only the activity decreases in females were significantly different from noninjected control, although the activity increases at 30–150 mg/kg were similar to the significant increases produced in males. Together the data suggested that triadimefon produced a biphasic effect on motor activity. The activity-decreasing effect of 300 mg/kg was also present at 24 and 48 hr in females. Males had recovered from the activity-increasing effects by 24 hr. No significant main effect or interaction was present at 6 days.

In the first 5-min interval, there was no effect of triadimefon on motor activity in either sex. Analysis of the temporal pattern of activity, however, revealed a significant effect on habituation (temporal distribution of activity within the session) at 20 min (males only) and 24 hr (both sexes) as reflected by a significant dose-by-time interval interaction. The effects of 150 mg/kg triadimefon on intrasession activity are shown in Fig. 6. From 30 min on, the groups diverged with activity levels of rats dosed with 150 mg/kg triadimefon being elevated when compared to vehicle or noninjected controls. This effect was significant only in males at this time point. At 20 min and 48 hr, the highest dose produced in females (only) significant decreases in activity throughout the session, with only a significant dose effect but no dose-by-interval interaction. At 24 hr, 300 mg/kg significantly decreased activity only during the first 9 intervals.

Triadimefon also affected the spatial distribution of activity in the figure-8 mazes (data not shown). In males there was a significant dose effect in the photocell counts in the figure-eight portion only (75 and 150 mg/kg) on the day of dosing. Thus the increase in overall activity at these doses was due to the increased activity in the figure-eight portion only. In females, the activity-suppressing dose, 300 mg/kg, decreased activity equally in both areas of the chamber at 20 min and 24 hr.

TABLE 2

RESPONSE RATES (% CONTROL ± S.E.M.) FOR PROBE EXPERIMENTS, COMPARING TRIADIMEFON TO AMPHETAMINE (1 AND 2 mg/kg) (TOP PORTION) AND ADMINISTERING DOUBLE INJECTIONS OF CHLORPROMAZINE 0.5 mg/kg AND TRIADIMEFON (10–56 mg/kg) (BOTTOM PORTION)

Triadimefon	Response Rate	Amphetamine	Response Rate
56 mg/kg	219.3 ± 50.9	1 mg/kg	211.0 ± 34.6
100 mg/kg	108.7 ± 47.2	2 mg/kg	114.2 ± 37.9
Saline and Triadimefon	**Response Rate**	**Chlorpromazine and Triadimefon**	**Response Rate**
vehicle	97.3 ± 8.3	vehicle	72.8 ± 4.4
10 mg/kg	167.8 ± 10.9	10 mg/kg	128.6 ± 9.3
30 mg/kg	244.9 ± 41.6	30 mg/kg	137.6 ± 24.5
56 mg/kg	168.8 ± 40.7	56 mg/kg	153.3 ± 27.3

N = 7 male rats, except following amphetamine 2 mg/kg where N = 6.

Experiment 3—Schedule-Controlled Behavior

Average overall rate of responding was 0.17 resp/sec (range: 0.06 to 0.25) for males, and 0.18 resp/sec (range: 0.07 to 0.31) for females. Under baseline conditions the fixed-interval schedule produced little or no responding during early portions of the interval, and a rapid rate of responding during later portions. Positive IOC values reflect this pattern of within-interval responding.

The effects of triadimefon on overall response rates and IOC values on the day of dosing are shown in Fig. 7. Unlike the motor activity experiments, there was no dose-by-sex interaction and thus the data for both sexes were pooled for analysis. Responding following 30 and 56 mg/kg was significantly increased, and larger doses produced a graded decrease in response rate. Examination of the individual data revealed that for each rat, the maximum response rate-increasing effect was seen at either 30 or 56 mg/kg.

The effects of triadimefon on temporal patterning of responding are also shown in Fig. 7. IOC values were unaffected by 10–30 mg/kg and were decreased uniformly by 56–200 mg/kg. A more detailed analysis of triadimefon's effects on response patterning is seen in Fig. 8. Decreases in IOC produced by 56 mg/kg were largely due to substantial increases in responding early in the interval. At 200 mg/kg, rate increases early in the interval were accompanied by decreases late in the interval. The comparable slopes of the functions shown in Fig. 8 are responsible for the equivalent decreases in IOC seen in Fig. 7 following 56 and 200 mg/kg.

Rats in this experiment were still affected to various degrees 1–3 days after a high (≥56 mg/kg) dose (data not shown). However, the time course of this performance disruption was very individualized, with a few rats taking up to a week to recover baseline responding. In addition, four of the six females required more than a month to recover from the highest dose.

Probe experiments. Table 2 presents the response rate data (as percent control) for doses of triadimefon alone, *d*-amphetamine alone, and combinations of triadimefon and chlorpromazine. Response rate data are presented for males only; IOC data showed essentially the same results. It is apparent that *d*-amphetamine and triadimefon produced similar response rate increases in this dose range. (Data for females were similar but more variable, since

some females required months to recover baseline responding following injection of the high dose of triadimefon.) The bottom portion of Table 2 shows the redetermination of effects of some triadimefon doses, with saline given 10 min earlier. The inverted U-shaped dose-response curve was again obtained. Chlorpromazine 0.5 mg/kg 10 min before triadimefon vehicle suppressed responding slightly, but this was not a significant difference (Student's *t*-test). Combinations of chlorpromazine 0.5 mg/kg and triadimefon showed a marked attenuation of the effects of 10 and 30 mg/kg triadimefon, but no change in the effect of 56 mg/kg triadimefon by chlorpromazine. Thus chlorpromazine appeared to antagonize some of triadimefon effects, but this interaction may be surmountable with higher doses of triadimefon.

DISCUSSION

Triadimefon produced marked disruptions of behavior in both male and female rats. Motor activity (males only) and operant responding (both sexes) were increased well over control levels at certain doses, and the temporal patterning of these behaviors was altered (both sexes). Arousal in the open field was increased as well as certain reactive measures (e.g., ease of handling, removal, and tail-pinch response). Various patterns of repetitive, stereotypic behavior were also observed along with self-mutilation of the digits and limbs. Thus these data suggest that triadimefon produces a characteristic syndrome of intoxication in rats that is unique among pesticides.

The present results agree well with other reports of triadimefon effects on motor activity (6,17). Crofton *et al*. (6) reported that intermediate doses of triadimefon produced hyperactivity in male rats whereas larger doses were without effect. The present experiment also showed that the highest dose of triadimefon decreased motor activity in female rats. Alterations in the temporal and spatial distribution of motor activity, similar to those shown by Crofton *et al*. (6), were also seen in both male and female rats.

There are many ways in which the effects of triadimefon resembled those produced by psychomotor stimulants (e.g., *d*-amphetamine, methylphenidate) whose actions are primarily mediated through catecholaminergic mechanisms (13, 16). For example, *d*-amphetamine increased activity levels in a figure-eight maze and altered the spatial pattern of activity (28). Triadimefon also increased overall rates of fixed-interval reinforced responding and altered the within-interval pattern of responding. The change in the temporal pattern of responding consisted of an increase in responding when it ordinarily occurred at a low rate early in the fixed interval, and a decrease in responding when it occurred at a high rate late in the interval. Similar effects were obtained in this experiment with *d*-amphetamine [see also (7) for review], and were reported by Sagvolden *et al*. (30) for methylphenidate.

Another pronounced effect produced by high doses of CNS stimulants are stereotypic movements. The syndrome of stereotypies produced by *d*-amphetamine, methylphenidate,and other stimulants has been described in detail [e.g., (9, 11, 25, 26)]. Triadimefon also produced the repetitive sniffing, pacing, and head movements often reported for *d*-amphetamine. These findings have recently been confirmed in another laboratory using an observational rating scale designed specifically for CNS stimulants (31). Autotomy, or self-mutilation, has been described following exposure to a number of chemicals including psychomotor stimulants [for review, see (15)]. Moreover, pemoline, an analog of methylphenidate, induced a persistent stereotyped biting of the digits and feet in rats (22), much like that seen following triadimefon.

Thus triadimefon produced many of the effects seen following acute exposure to psychomotor stimulants such as *d*-amphetamine and methylphenidate. There is, however, at least one exception. High doses of *d*-amphetamine and most other stimulants produce an increase in body temperature [e.g., (8,18)], whereas triadimefon produced dose-related hypothermia. Triadimefon may not be entirely analogous to CNS stimulants, and may have some additional actions.

Sex differences emerged in the time-course and dose-response characteristics of triadimefon, although these differences varied across measures. For some measures, e.g., operant responding, stereotypies, and equilibrium, males and females were both affected from 0.5 to 48 hr after dosing. For motor activity and some other FOB measures (e.g., body temperature) males had recovered from triadimefon effects by the next day. This time course has also been previously described, leading to assumptions that triadimefon is relatively short-acting (6). In contrast, many effects in females were more prolonged. For example, motor activity decreases were significant in females even at 48 hr following the high dose of triadimefon, whereas males were at control levels at 24 hr. In some cases, the magnitude of response to triadimefon in females was greater than that in males. For instance, females consistently showed more self-mutilation than males. Motor activity and some sensory reactions were decreased following 300 mg/kg in females, in contrast to the biphasic effects seen in males. This sex difference in sensitivity and time course could be due to the different rates of metabolism and/or excretion of triadimefon. Triadimefon is metabolized in another bioactive triazole, triadimenol (10), which is equally potent as the parent compound in producing hyperactivity in mice (17). The relative contribution of these two triazoles, and any other active metabolite(s), to the syndrome of intoxication produced by triadimefon is still unknown.

This is the first report of the effects of triadimefon using the FOB and schedule-controlled operant behavior, as well as the first study of triadimefon in female rats. The data indicate that triadimefon produced an array of overt as well as subtle behavioral changes, with females somewhat more sensitive than males. Recovery was evident in some measures the day after dosing, but many effects of high doses ($\geq$100 mg/kg) were more prolonged, especially in females. The syndrome of hyperactivity, stereotypy, increased excitability, and changes in patterns of behavior was similar to those reported for CNS stimulants. Further research is needed to both explore additional effects of triadimefon as well as determine whether similar effects are produced by other triazole fungicides.

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